



# Millicomposting: Sustainable technique for obtaining organic compost for the cultivation of broccoli seedlings

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## ABSTRACT

Millicomposting is a little-known technique in Brazil and especially in the world. It consists of the use of millipedes, which through their millipede feeding activity, enhance the fragmentation of agricultural and urban waste. It is through the accumulation of its fecal pellets that the formation of the organic compost, which we call millicompost, occurs. Given the need to increasingly prioritize the use of sustainable substrates, the objective of this work was to evaluate the efficiency of millet in the production of broccoli seedlings. The substrates used were: millicompost obtained after 180 days of millicomposting and the commercial substrate Biomix<sup>®</sup>organic. The substrates were characterized as to their physicochemical, chemical and physical properties. Shoot and root dry mass (g), plant height (cm), number of leaves, seedling vigor and clod stability were evaluated. Millicompost showed greater total macronutrients supply around 201, 138, 85, 293 and 29% for nitrogen, phosphorus, potassium, calcium and magnesium, respectively. Concerning to physical properties, the millicompost showed higher water retention capacity and lower volumetric density. These results reflected in an increase of 217% in the accumulation of shoot dry mass and 148% in the root dry mass, promoting 36% more clod stability, providing the formation of broccoli seedlings with height two times greater than the seedlings developed in the commercial substrate Biomix<sup>®</sup>organic. The millicomposting represents a new alternative for recycling organic waste to obtain organic substrate for producers, as it provides more vigorous seedlings for transplanting in production sites.

## 1. Introduction

Organic waste has become a major focus of research due to the threat it poses to the environment and human health in modern society. The global production of agricultural waste is more than hundreds of megatons per year and a large part of this agricultural waste is disposed of inappropriately or burned directly, further intensifying global warming and air pollution (Zhang et al., 2016).

Composting is a biological process that acts on the transformation of the organic matter present in the residues into humidified material, where the generated compost can be used as organic fertilizer in agriculture, vegetable gardens and gardens (Antunes et al., 2021). In addition, to its low production cost, it is an environmentally friendly

technique for the sustainable processing of this waste (López-González et al., 2015). From classic composting, in which microbial activity is of decisive importance, to composting mediated by invertebrates from the soil fauna, such as vermicomposting and millicomposting, different mixtures, practices and managements have been proposed with the objective of improving the efficiency of composting and the quality of the compost produced.

Millicomposting is a new biotechnology, little known and environmentally friendly, which provides the biotransformation of plant residues into stable organic matter, which is promoted by the activity of diplopods, popularly called millipedes. The diplopods species *Trigoniulus corallinus* (Fig. 1) are viable for millicomposting, as it has a pantropical distribution, occurring widely in different agricultural environments,

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Fig. 1. Diplopods of the species *Trigoniuulus corallinus* (Gervais) above the formation of millicompost.

and is easily recognized by its distinct red color (Antunes, 2017).

Diplopods are widely distributed in tropical, subtropical and temperate regions, playing an important role in improving soil fertility. These organisms are able to mobilize nutrients trapped in litter and enrich the soil with nitrogen (N), carbon (C), calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K), in microcosm situations (Antunes et al., 2019). This is due to the capacity of litter consumption associated with a high microbial activity present in the faecal pellets of diplopods. When the litter passes through the digestive tract, this material is crushed, which increases its specific surface, moistened and enriched with microorganisms (Aquino and Correia, 2005). Diplopods can metabolize 0.3–7% of the ingested material and, when excreting microbial activity, continue in their faecal pellets, which increases the bioconversion of plant residues (Ambarish and Sridhar, 2013).

Although millipedes are confused by many people as a type of earthworm, they are not earthworms. The earthworms belong to the Oligochaeta class and millipedes to the Diplopoda class. In this sense, earthworms do not have the ability to crush fibrous and recalcitrant residues in nature, which makes millipedes have advantages over them in this regard. It is also noteworthy that there are regions that do not have manure available for the production of vermicompost, making the production of millicompost more viable. Lignocellulosic residues and millipedes are more easily found, making the millicomposting a viable activity in rural and peri-urban areas.

The final product of millicomposting is millipede humus, which has been called millicompost (Antunes et al., 2021). The use of organic waste as suppliers of nutrients and support to compose substrates may represent an alternative to reduce the cost of production of vegetable seedlings (Silva Júnior et al., 2014).

The use of the substrate is essential for the production of vegetable seedlings and that it must bring together physical, chemical and biological characteristics that ensure good plant development in the seedling phase and in the subsequent development in the production beds. Thus, the aim of this work was to evaluate the efficiency of millicompost obtained from agricultural and urban residues in the production of broccoli seedlings and to disseminating the millicomposting technique as a new option of organic substrate from renewable sources to society generally.

## 2. Material and methods

### 2.1. Millicomposting process and obtaining the millicompost

The millicomposting process was conducted in the experimental area

of the Integrated System of Agroecological Production - SIPA, located at Fazendinha Km 47, Seropédica-RJ. The region's climate is hot and humid, classified as Aw, with rainfall concentrated from November to March and an average annual rainfall of 1213 mm (Oliveira Júnior et al., 2014). For this, a concrete ring with a height of 0.5 m and a width of 1 m was used, with the capacity to receive 400 L of waste (Fig. 2). In the first stage, the residues were quantified and deposited inside the ring. Millicomposting was established from the mixture of residues from *Bauhinia* sp. (cow paw leaves), *Paspalum notatum* (grass clippings), *Musa* sp. (banana leaves) and chopped cardboard. The proportion of materials was 160 L, 120 L, 80 L and 40 L, respectively. Then they were mixed to make the mixture very homogeneous, added to the ring and wetted (Fig. 2). The proportion of waste was based on a preliminary study, which consisted of observing the consumption of different agricultural and urban waste by millipedes (Antunes et al., 2019).

In the second stage, the ring received an approximate amount of 2.2 L of millipedes (*Trigoniuulus corallinus*), which is equivalent to a population of approximately 3960 adult individuals (Fig. 2), collected manually in earthworm beds, in compost pans and on grass containing fresh cuttings. The ring remained covered with a protective screen, whose function was to prevent the millipedes escaping when climbing the wall of the ring or to prevent the entry of something undesired during the millicomposting process (Fig. 2).

During the entire millicomposting there was a need to observe the moisture content of the material contained in the rings. Maintenance of moisture was carried out with the addition of water via hose can (approximately 4 L) weekly or when necessary, in order to keep it around 50–60%. The purpose of which was to provide a favorable environment for the survival of the millipedes and the continuity of the millicomposting process.

The millicompost was obtained at 180 days after starting the above process. The residues were removed and sieved in a 2 mm mesh (Fig. 2) and stored in plastic bags, and later destined for the production of vegetable seedlings.

### 2.2. Characterization of the physical, physico-chemical and chemical properties of the millicompost

The physical characteristics evaluated in the substrates were: macroporosity, microporosity, total porosity, water retention capacity and volumetric density, obtained by the table method of tension, using metal rings of 100 mL and tension of 60 cm of water column (Brasil, 2008; Teixeira et al., 2017). To evaluate physical-chemical characteristics the substrates, pH analyzes were performed in a distilled water solution (5: 1 v/v) and the electrical conductivity was determined in the same aqueous extract obtained for pH measurement, according to with the method described by Brasil (2008). As for chemical properties, samples of each substrate were sent to the Agricultural Chemistry Laboratory of Embrapa Agrobiologia, to determine the total levels of P, K, C and Mg levels were determined in aqueous acid extract after their own nitric-perchloric digestion, as Teixeira et al. (2017). The determination of the levels of N and C was done in the elementary analyzer (CHN) (Nelson and Sommers, 2015). Triplicates were performed for all of the aforementioned analyzes.

### 2.3. Production of broccoli seedlings

The experiment was carried out in a greenhouse on the premises of Embrapa Agrobiologia, Seropédica-RJ and the substrates used in the experiment were as follows: millicompost obtained with 180 days of millicomposting (item 2.1) and the commercial substrate Biomix® organic.

To evaluate the efficiency of the millicompost as substrate, early Piracicaba broccoli seeds (*Brassica oleracea* var. *Italica*) were sown in expanded polystyrene trays of 200 cells filled with the aforementioned substrates (each cell holds 12.5 mL of substrate). The experimental



**Fig. 2.** Sequential scheme of the millicomposting process carried out by millipedes of the species *Trigonulius corallinus* until obtaining the millicompost for use as a substrate in the production of vegetable seedlings.

design adopted was completely randomized, with four replications, each repetition consisting of 10 seedlings removed at random from each tray. At 23 days after sowing, the following phytotechnical parameters were evaluated: shoot dry mass (g), root dry mass (g), plant height (cm), number of leaves, seedling vigor and clod stability.

The seedling vigor is a methodology adapted from [Franzin et al. \(2005\)](#), classifying as: note 1: excellent vigor, number of leaves  $\geq 4$ , height greater than 5 cm and visual absence of nutritional deficiency; note 2: good vigor, number of leaves  $\geq 4$ , height  $\geq 5$  cm and yellow beginning not prominent in the basal leaves; note 3: regular vigor, number of leaves  $\geq 4$ , height  $\geq 5$  cm; nutritional deficiency expressed by a prominent yellowing that extends beyond the basal leaves or other intrinsic symptom; note 4: poor vigor, well-defined nutritional deficiency, expressed by height problems ( $\leq 5$  cm), reduced number of leaves ( $\leq 4$  leaves) and intense yellowing or other intrinsic symptom.

Clod stability is a methodology adapted from [Antunes et al. \(2018\)](#), classifying as: note 1: low stability, 50% or more of the clod is retained in the container when the seedling is removed and the clod does not remain cohesive; note 2: between 30 and 50% of the clod is retained in the container when the seedling is removed, but the clod does not remain cohesive; note 3: regular, between 15 and 30% of the clod is retained in the container when the seedling is removed, but it does not remain cohesive; note 4: good stability, the clod is completely detached from the container with up to 90% cohesion and maximum loss of up to 10% of the substrate; note 5: excellent stability, the clod is completely detached from the container and more than 90% of it remains cohesive, with losses of less than 10% of substrate.

After data collection and tabulation, they were subjected to analysis of variance by the F test ( $p \leq 0.05$ ), using the SISVAR statistical program ([Ferreira, 2014](#)).

### 3. Results

#### 3.1. Physico-chemical, chemical and physical properties of substrates

The pH value of the millicompost was significantly higher than that found in the commercial substrate Biomix<sup>®</sup>organic. The electrical conductivity (EC) also showed a significant difference between the substrates, with the EC of Biomix<sup>®</sup>organic being 66.7% lower. The carbon/nitrogen ratio (C/N) was 2.7 times higher in the Biomix<sup>®</sup>organic

**Table 1**

Physico-chemical, chemical and physical characteristics of the organic substrates used in the production of broccoli seedlings.

Parameters	Substrates	
	Millicompost	Commercial
<b>Physico-chemical characteristics</b>		
pH	7.92 a	7.27 b
EC (dS m <sup>-1</sup> )	0.84 a	0.28 b
<b>Chemical characteristics</b>		
C/N ratio	16.84 b	46.56 a
Carbon (g kg <sup>-1</sup> )	354 a	325 b
Nitrogen (g kg <sup>-1</sup> )	21.02 a	6.98 b
Phosphorus (g kg <sup>-1</sup> )	3.40 a	1.43 b
Potassium (g kg <sup>-1</sup> )	4.83 a	2.61 b
Calcium (g kg <sup>-1</sup> )	37.41 a	9.52 b
Magnesium (g kg <sup>-1</sup> )	4.63 a	3.60 b
<b>Physical characteristics</b>		
Total porosity (%)	78.97 a	78.80 a
Macroporosity (%)	12.50 b	18.34 a
Microporosity (%)	66.47 a	60.46 b
Water holding capacity (mL 50 cm <sup>-3</sup> )	33.24 a	30.23 b
Volumetric density (kg m <sup>-3</sup> )	290 b	400 a

substrate, whereas the total carbon content present in this substrate was lower than that found in the millicompost (Table 1). As for the total content of macronutrients, the millicompost showed significantly higher averages of about 201, 138, 85, 293 and 29% for nitrogen, phosphorus, potassium, calcium and magnesium, respectively (Table 1).

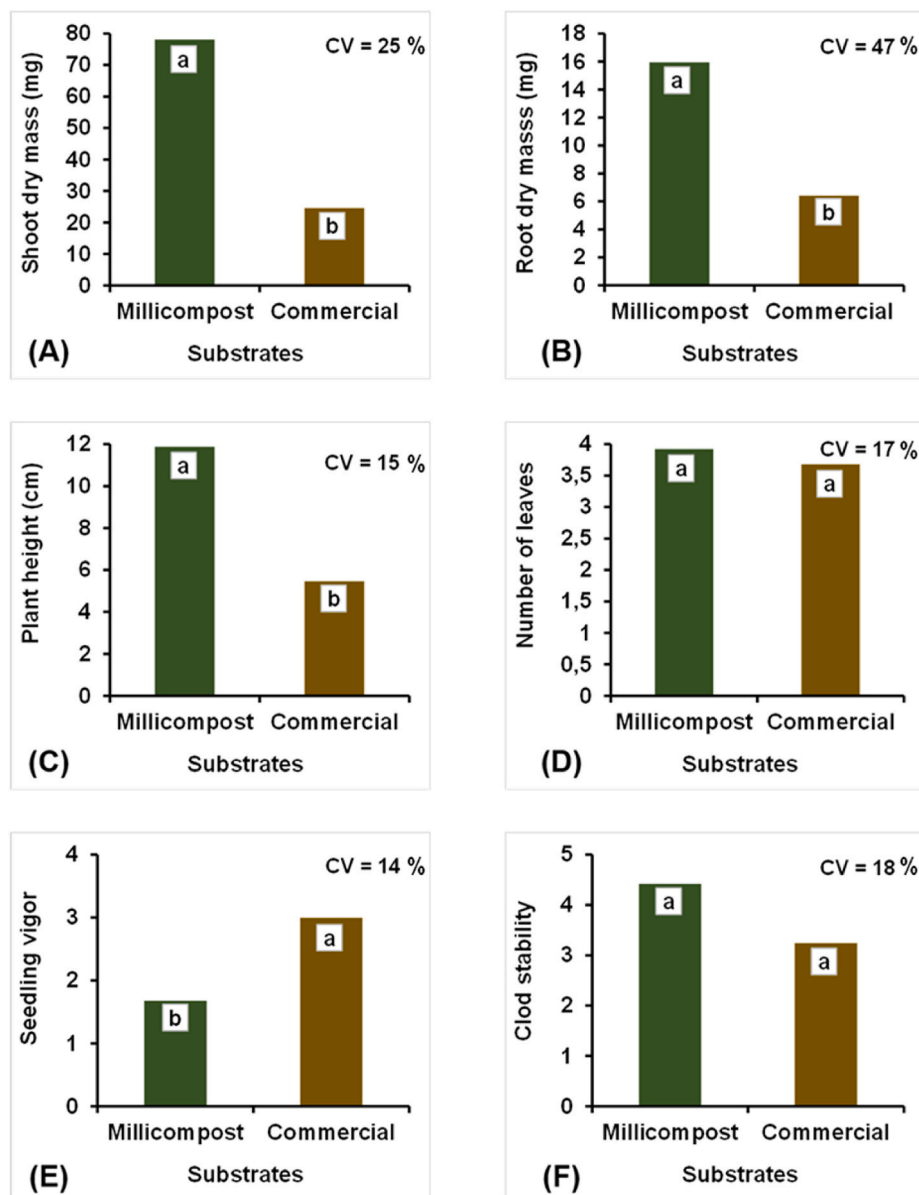
The percentages of macropores and micropores present in the substrates were significantly different from each other. The millicompost exhibited 31.84% less macropores in relation to the Biomix<sup>®</sup>organic substrate, which in turn presented 9.94% less micropores in relation to the millicompost (Table 1). The total porosity did not differ between the substrates, showing values similar to each other (Table 1). The water retention capacity followed the same pattern as the microporosity, and the millicompost exhibited significantly higher percentages in relation to the Biomix<sup>®</sup>organic substrate (Table 1). As for the volumetric density, the millicompost was 27.5% less dense than Biomix<sup>®</sup>organic substrate (Table 1).

Values followed by the same letter on the line do not differ by the F

test ( $p \leq 0.05$ ).

### 3.2. Production of broccoli seedlings

The broccoli seedlings produced in the millicompost showed significantly higher averages for all phytotechnical parameters (Fig. 3 and Fig. 4), except for the number of leaves, where the commercial substrate exhibited a 6.52% higher average than the millicompost (Fig. 3-D). In this way, the percentage differences of the other phytotechnical parameters in the seedlings originating from the millicompost were 217, 148, 117 and 36% higher for shoot dry mass (MSPA), root dry mass, plant height and note the clod stability, respectively. As for the seedling vigor score, the millicompost provided seedling vigor close to note 1, which was 44% lower than the commercial substrate seedlings, whose average score was 3 (Fig. 3-E).



Values followed by the same letter on the bar do not differ by the F test ( $p < 0.05$ ).

**Fig. 3.** Average values of shoot dry mass (A); dry root mass (B); plant height (C); number of leaves (D); seedling vigor (E) and clod stability (F) of broccoli seedlings produced on organic substrates at 23 days after sowing. Values followed by the same letter on the bar do not differ by the F test ( $p < 0.05$ ).



Fig. 4. Morphological differences between broccoli seedlings produced in organic substrates at 23 days after sowing. On the left, the seedling developed from millicompost (A) and on the right the seedling from the commercial substrate (B).

## 4. Discussion

### 4.1. Physical-chemical, chemical and physical properties of substrates

Kratz and Wendling (2013) report that when it comes to the use of organic substrates, without the addition of soil in their composition, the recommendation is to work with a pH range of 4.4–6.2. The two substrates showed pH values above that recommended by the authors, ranging from 7.27 to 7.92 (commercial substrate and millicompost, respectively). However, these values were not able to negatively influence the development or the quality of seedlings of the millicompost (Fig. 4-A). The seedlings from the commercial substrate were affected by the lack of nutrients (Fig. 4-B).

EC values between 2.0 and 4.0 dS m<sup>-1</sup> are considered high for substrates, values from 1.0 to 2.0 dS m<sup>-1</sup> are normal and less than 1.0 dS m<sup>-1</sup> are considered low (Araújo Neto et al., 2009), therefore, the two substrates had low concentrations of salts. It is interesting that the EC does not present high values, because the excess of soluble salts in the substrate can interfere in the germination or in the other stages of development of the plants cultivated in the substrates.

Plants remove water from the substrate or soil when the imbibition forces of the root tissues are greater than the forces with which the water is retained in the substrate or soil, that is, the maintenance of a water potential gradient favorable to the entry of water in the root cells. In substrates with higher electrical conductivity, the soluble salts in the solution increase the water retention forces due to the osmotic effect, thus causing a reduction in water absorption by the plant. The increase

in osmotic pressure, caused by the excess of soluble salts, may reach a level where the plants will not have sufficient suction forces to overcome this osmotic pressure and, as a result, the plant will not absorb water, even in wet substrate/soil (Dias et al., 2016).

According to Pascual et al. (2018), the C/N ratio has been used as an index to determine the stability of the organic substrate, since it was established that a C/N 15 ratio allows plants to absorb nitrogen without it leaching as nitrate and the C/N ratios above 15 represent values at which nitrogen is immobilized. Another point to be considered is regarding the N total content present in the substrate, since low values associated with certain mixtures of raw materials will cause the C/N ratio to be higher. This is the case with the commercial substrate, which consists of several mixtures, including pine bark, which has a higher C/N ratio (Antunes et al., 2021). Normative instruction No. 61 of the Ministry of Agriculture, Livestock and Supply (Brasil, 2020) highlights that the C/N ratio cannot be greater than 20 for organic composts, thus, the millicompost meets the requirements proposed to be used as substrate or compost in agriculture.

The highest content of nutrients present in the millicompost was observed for Ca and N, which were 292 and 201% higher, respectively, in relation to the contents observed in the commercial substrate Bio-mix® organic. Antunes et al. (2016) highlight that the activity of micro-organisms present in the millipede intestine are capable of making available the nutrients trapped in the plant material that they consume and part of them are eliminated in the feces. The digestive system of soil invertebrates contains evolutionarily diverse microbes and this implies the occurrence of several associations, one of which is responsible for the decomposition of organic matter and the nutrient cycle (Correia et al., 2018). In addition to the benefits observed by the millipede activity, the greater nutrient richness of the millicompost may result from the residues used during millicomposting, since the grass has 2% N and the cow's foot 3.5% Ca in its tissues (Antunes et al., 2019), together constituting 70% of the mixture.

The physical properties of the substrates are also able to influence positively or negatively on plant development in containers. Gonçalves and Poggiani (1996) established appropriate ranges for the physical characteristics of the substrates. For total porosity, the authors consider adequate percentages ranging from 75 to 85%, with both substrates being 78%. As for macroporosity, all substrates showed values above the range considered adequate (45–55%), whereas for microporosity, both presented percentages below the adequate (35–45%). Adequate levels of water holding capacity must be between 20 and 30 mL 50 cm<sup>-3</sup>, thus, the commercial substrate meets the proposed range, however the millicompost exhibited a 10% higher water holding capacity. According to Sá et al. (2020), in general, the substrates constituted by organic residues present a predominance of micropores at the expense of macropores, leading to greater water retention, corroborating the physical properties of the millicompost.

Fermino (2014) establishes as a reference for substrates used in trays, volumetric density values between 100 and 300 kg m<sup>-3</sup>. Thus, only the millicompost was found to be within the standard, while the commercial substrate had a density 33% higher than the range proposed by the author. Maggioni et al. (2014) highlight those substrates with lower densities provide greater porosity, better drainage and less physical restrictions on plant growth and development.

### 4.2. Production of broccoli seedlings

Souza and Resende (2014) explain that in the production of broccoli seedlings in trays it is essential to use quality organic substrates, capable of providing the nutrients necessary for plant nutrition and development. Considering that the volume of each cell is 16 cm<sup>3</sup>, it is ensured that the millicompost adequately supplied water, air and nutrients, in addition to providing excellent support to the root system of broccoli seedlings. These conditions reflect on obtaining excellent quality broccoli seedlings, whose phytotechnical parameters were higher than those

found in seedlings from the commercial substrate.

According to [Pereira et al. \(2020\)](#), there is no current information on the adequate levels of nutrients in plant substrates. However, the macronutrients present in the millicompost were essential for obtaining vigorous seedlings, mainly due to nitrogen and phosphorus. Nitrogen is the nutrient that influences most physiological processes that occur in plants, such as protein synthesis and photosynthesis, being the most limiting nutrient for biomass production ([Yong et al., 2010](#)). Phosphorus has important structural functions for plant development, participating in photosynthesis, respiration, cell division and growth, and especially in energy supply (ATP), which results in greater growth and initial development of plants, especially of the root system ([Vieira et al., 2015](#)).

[Ramanathan and Alagesan \(2012\)](#) when comparing the efficiency of vermicompost and millicompost generated at 60 days, from flower remnants, in the production of pepper in pots, obtained the best parameters of plant height, number of leaves, leaf area, number and fruit weight in the millicompost, which had higher total contents of N, P, K and Ca. Results similar to those observed in this work were also recorded by [Antunes et al. \(2021\)](#). The authors noted that millicompost obtained from different vegetable residues, locations and species of millipedes, resulted in the production of lettuce seedlings with higher plant height averages, number of leaves and seedling vigor, in relation to the commercial substrate Biomix® organic. [Antunes et al. \(2016\)](#) too found that the contents of nutrients such as Ca, Mg and P, as well as the physicochemical and physical characteristics of the compost generated by diplopods made the millicompost efficient as a substrate in the production of lettuce seedlings, corroborating with the results achieved in this work ([Fig. 4-A](#)).

Although the experiment did not proceed with the transplanting phase to the production field, the greater amount of dry matter observed in the seedlings developed from millicompost, as well as its entire root structure, presented by the good stability of the clod ([Fig. 4-A](#)), allowing us infer that they would have an excellent agronomic performance.

The value-added bio-product of the composting process contributes to the improvement of soil properties and plant growth in an ecologically correct way. Lignocellulosic residues can be the driving force for the production of nutrient-enriched compost, contributing significantly to agriculture and the environment, as they are inexpensive, available all year round and are widely distributed worldwide ([Harindintwali et al., 2020](#)). And through millicomposting, the millicompost can contribute to the maintenance of nutrient recycling within rural or urban properties, reducing the need to purchase inputs, especially with regard to the production of seedlings in containers.

Earthworm humus is already recognized for its great potential to be used in the formulation of substrates used in the production of seedlings in organic production systems, mainly due to the ease of access to raw material from livestock activities ([Steffen et al., 2010](#); [Oliveira et al., 2013](#)). Substrates formulated with residues from the activity of diplopods are little known, however, they also have great potential for the production of lettuce seedlings, because the millipedes they are easily found for capture in humid areas with an accumulation of plant material (litter), where they act in their decomposition.

## 5. Conclusions

Millicompost contributes to an increase in nutrient content of about 201, 138, 85, 293 and 29% for nitrogen, phosphorus, potassium, calcium and magnesium, respectively. It showed greater water retention capacity and lower volumetric density. These results reflected an increase of 217% in the accumulation of shoot dry mass and 148% in the dry mass of the root mass, showing 36% more clod stability, which reflected in the production of broccoli with height twice as high as compared to commercial substrate. In addition, the millicomposting represents a new alternative for recycling organic waste to obtain substrate. This technique will ensure producers obtain more vigorous seedlings for transplanting in production sites, combining waste

recycling with savings in the acquisition of commercial substrates or raw material for its formulation.

The biggest restriction to work with these animals is the limitation of humidity and reduced temperatures, that like as all arthropods, especially in winter, when these animals practically disappear or have their biological activity reduced. Unlike the earthworms, whose reproduction and management are well known, the management of millipedes requires more studies so that there is less and less need to collect them in vermicompost beds, stabilized compost piles or areas with accumulation of lignocellulosic organic material. During the millicomposting process, the millipedes mate and thus obtain many offspring, but few reach adulthood, because the life strategy of arthropods is to release a large number of individuals into the environment to colonize the sites, by these are known as strategists. “r”.

Thus, more studies should be carried out in order to create environments, strategies and differentiated food supply for millipedes, in order to obtain better reproduction and survival rates in the adult stage of these animals.

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## CRedit authorship contribution statement

**Luiz Fernando de Sousa Antunes:** Conceptualization, Methodology, Investigation, Formal analysis and data curation, Visualization, Supervision, Writing—original draft preparation and Writing—review and editing. **Letícia Spolador Fernandes:** Investigation, Visualization and Writing—review and editing. **André Felipe de Sousa Vaz:** Investigation, Visualization and Writing—review and editing. **Maura Santos Reis de Andrade da Silva:** Visualization and Writing—review and editing. **Talita dos Santos Ferreira:** Visualization and Writing—review and editing. **Dieini Melissa Teles dos Santos:** Visualization and Writing—review and editing. **Maria Elizabeth Fernandes Correia:** Resources and Funding acquisition.

## Data availability statements

All data generated or analyzed during this study are included in this published article.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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